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PTO/SB/05 (4/98)
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	E-1658
First Inventor or Application Identifier	David A. Holman et al.
Title	Method and Apparatus for Packed Column...
Express Mail Label No.	EE797446574US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231**

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)*
   (Submit an original and a duplicate for fee processing)
2. [X] Specification [*Total Pages* 33]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings (*if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 U.S.C. 113)* [*Total Sheets* 8]
4. Oath or Declaration [*Total Pages* 4]
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d))
   (for continuation/divisional with Box 16 completed)
   i. [ ] DELETION OF INVENTOR(S)
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

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5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission
   (if applicable, all necessary)
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [X] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [X] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [X] Return Receipt Postcard (MPEP 503)
    (Should be specifically itemized)
13. [X] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
14. [ ] Certified Copy of Priority Document(s)
    (if foreign priority is claimed)
15. [ ] Other: .....

16. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment:*

[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: _____/_____

Prior application information: *Examiner* _____ *Group / Art Unit:* _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

[ ] *Customer Number or Bar Code Label* *(Insert Customer No. or Attach bar code label here)* *or* [X] *Correspondence address below*

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Name (Print/Type)	Paul W. Zimmerman	*Registration No. (Attorney/Agent)*	34,761
Signature	Paul W. Zimmerman	*Date*	23/OCT/23

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These are the fees effective October 1, 1997.
Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12.
See 37 C.F.R. §§ 1.27 and 1.28.

TOTAL AMOUNT OF PAYMENT ($) 477.00

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	David A. Holman et al.
Examiner Name	
Group / Art Unit	
Attorney Docket No.	E-1658

METHOD OF PAYMENT (check one)

1. [X] The Commissioner is hereby authorized to charge indicated fees and credit any over payments to:

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Deposit Account Name: Battelle Memorial Institute, Pacific Northwest Division

[X] Charge Any Additional Fee Required Under 37 C.F.R. §§ 1.16 and 1.17
[ ] Charge the Issue Fee Set in 37 C.F.R. § 1.18 at the Mailing of the Notice of Allowance

2. [ ] Payment Enclosed: [ ] Check [ ] Money Order [ ] Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	790	201	395	Utility filing fee	395
106	330	206	165	Design filing fee	
107	540	207	270	Plant filing fee	
108	790	208	395	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 395.00

2. EXTRA CLAIM FEES

	Extra Claims		Fee from below		Fee Paid
Total Claims	20 -20** =	0	X	=	
Independent Claims	5 - 3** =	2	X 41	=	82
Multiple Dependent				=	

**or number previously paid, if greater; For Reissues, see below

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	22	203	11	Claims in excess of 20
102	82	202	41	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	82	209	41	** Reissue independent claims over original patent
110	22	210	11	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 82.00

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	950	217	475	Extension for reply within third month	
118	1,510	218	755	Extension for reply within fourth month	
128	2,060	228	1,030	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,320	241	660	Petition to revive - unintentional	
142	1,320	242	660	Utility issue fee (or reissue)	
143	450	243	225	Design issue fee	
144	670	244	335	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	790	246	395	Filing a submission after final rejection (37 CFR 1.129(a))	
149	790	249	395	For each additional invention to be examined (37 CFR 1.129(b))	

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Other fee (specify) _____

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SUBTOTAL (3) ($) -0-

SUBMITTED BY				Complete (if applicable)	
Typed or Printed Name	Paul W. Zimmerman			Reg. Number	34,761
Signature	Paul W. Zimmerman	Date	98/oct/23	Deposit Account User ID	005055

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Express Mailing Label # EE797446574US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
DA Holman, CJ Bruckner-Lea,	)	
FJ Brockman, and DP Chandler	)	
	)	
	)	
For: METHOD AND APPARATUS FOR	)	Our Ref. No: E-1658
PACKED COLUMN SEPARATIONS	)	
AND PURIFICATIONS	)	Date: October 23, 1998

CERTIFICATE OF MAILING

Box PATENT APPLICATION
Commissioner of Patents and Trademarks
Washington, DC 20231

Dear Sir:

The undersigned hereby certifies that the attached:

[X] Patent Application of 33 pages, and
[X] Drawings of 8 Sheets
[X] Fee Transmittal (2 ea.)
[X] Assignment / Cvr. Sheet
[X] Combined Declaration and Power of Attorney
[X] Small Entity
[ ] Information Disclosure
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Janice K. Laybourn
Signature

10/23/98
Date of Deposit

Janice K. Laybourn K1-53
Intellectual Property Services
Battelle Memorial Institute
Pacific Northwest Laboratories
P.O. Box 999
Richland, WA 99352
(509) 375-2655

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PATENT

File No. E-1658

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: David A. Holman, Cynthia J. Bruckner-Lea, Fred J. Brockman, and Darrell P. Chandler

Serial or Patent No.: _____

Filed or Issued: _____

For: METHOD AND APPARATUS FOR PACKED COLUMN SEPARATIONS AND PURIFICATIONS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(d)) -

I hereby declare that I am an official empowered to act on behalf of the entity identified below:

NAME: Battelle Memorial Institute
Pacific Northwest Division
ADDRESS: Post Office Box 999, Richland, WA 99352

TYPE OF ENTITY:

[X] Nonprofit Organization (37 CFR 1.9(e))
Nonprofit Scientific or Educational Under Statute of State of the United States of America
(Name of State Ohio)
(Citation of Statute Sections 1719.01 and 1719.05, Rev. Code of Ohio)
[ ] Small Business (37 CFR 1.9(d))
[ ] Independent Inventor (37 CFR 1.9(c))

I hereby declare that the entity identified above qualifies as such as defined in [ ] 37 CFR 1.9(c) [ ] 37 CFR 1.9(d) [X] 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled METHOD AND APPARATUS FOR PACKED COLUMN SEPARATIONS AND PURIFICATIONS by inventor(s) David A. Holman, Cynthia J. Bruckner-Lea, Fred J. Brockman, and Darrell P. Chandler described in

[ ] application executed _____

[X] specification filed herewith

[ ] application serial no. _____, filed _____

[ ] patent no. _____, issued _____.

I hereby state that rights under contract or law have been conveyed to and remain with the entity with regard to the above identified invention.

If the rights held by such entity are not exclusive, each individual, concern or organization having rights in the invention must file separate statements as to their status as small entities (37 CFR 1.27) and that no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:

NAME NONE
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[ ] INDEPENDENT INVENTOR [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

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NAME OF PERSON SIGNING Paul W. Zimmerman
TITLE OF ORGANIZATION Contracting Officer, Pacific Northwest Division, Battelle Memorial Institute
ADDRESS OF PERSON SIGNING Post Office Box 999, Richland, WA 99352

SIGNATURE Paul W. Zimmerman DATE 98/OCT/23

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION FOR LETTERS PATENT

* * * * *

METHOD AND APPARATUS FOR PACKED COLUMN SEPARATIONS AND PURIFICATIONS

* * * * *

INVENTORS

David A. Holman
Cynthia J. Bruckner-Lea
Fred J. Brockman
Darrell P. Chandler

ATTORNEY'S DOCKET NO. E-1658

METHOD AND APPARATUS FOR PACKED COLUMN SEPARATIONS AND PURIFICATIONS

This invention was made with government support under Contract DE-AC06-76RLO 1830 awardee by the U.S. Department of Energy. The Government has certain rights in the invention.

TECHNICAL FIELD

The invention pertains to a method and apparatus for column-based separation, and for forming and utilizing packed columns of beads. The present invention is particularly useful for biological sample separations.

BACKGROUND OF THE INVENTION

Column-based separations are frequently used for selectively removing components from mixtures. A first step in utilizing column-based technology is to form a column. Such can be accomplished within a column chamber. An exemplary prior art column chamber **10** is illustrated in **Fig. 1**. Column chamber **10** comprises a longitudinal tubular section **12** having ends **14** and **16**. An inlet **18** is provided at end **16**, and an outlet **20** is provided at end **14**. Outlet **20** is obstructed by a porous filter **22**. Filter **22** can comprise, for example, a porous fritted glass or ceramic material.

A packed column is formed within chamber **10** by flowing a slurry comprising a mixture of matrix material **15** and carrier fluid **17** into inlet **18**. Matrix material **15**

consists of particulates, typically spherical, with extractive or chemically selective surface. Filter **22** is permeable to the carrier fluid and impermeable to the matrix material. Accordingly, as the slurry is flowed into column chamber **10**, matrix material **15** stacks against filter **22** to form a packed column **19** within tubular portion **12**.

The composition of carrier fluid **17** and matrix material **15** vary depending on the components that are intended to be separated by the packed column, and on the mixtures (samples) within which such components are found. Materials that can be separated utilizing column-based systems are, for example, biological materials, such as nucleic acids. For instance, Tepnel Life Sciences supplies a resin in the form of polymeric micro-beads in diameters of approximately 60-100 micrometers which are covalently linked to specific oligonucleotide capture probes. Such micro-beads can be utilized for selective purification of nucleic acid fragments from a biological sample. Biological sample or components that may be purified on resins such as micro-beads include but are not limited to nucleic acids, peptide nucleic acids, antibodies, receptors, proteins, ligands, cells, viruses, and combinations thereof. For purposes of interpreting this disclosure and the claims that follow, the term "nucleic acid" is defined to include DNA nucleotides and RNA nucleotides, as well as any length polymer comprising DNA nucleotides or RNA nucleotides. Prior art resin includes glass, polymer (e.g. sepharose, polystyrene, etc.), metal, ceramic and combinations thereof.

Other matrix materials are Sr-resin, TRU-resin, and TEVA-resin, all of which can be obtained from EIChrom Industries, Inc., of Darien, Illinois. Such matrix materials can

have particle sizes in the range of, for example, 20-100 micrometers. Sr-resin, TRU-resin, and TEVA-resin can be used for, for example, selectively retaining radioactive materials. Specifically, Sr-resin can selectively retain strontium, TRU-resin can selectively retain americium, and TEVA-resin can selectively retain technetium. Slurries utilized for forming packed columns of Sr-resin, TEVA-resin, or TRU-resin can comprise, for example, 0.074 gram/mL of Sr-resin in 3 M HNO_3; 0.142 grams/mL of TEVA-resin in 4 M HNO_3; or 0.076 grams/mL of TRU-resin in 0.1 M HNO_3, respectively.

In addition to the above-discussed exemplary uses for column-based separations, numerous other applications for column-based separations are known to persons of ordinary skill in the art. The column-based separations generally have in common that a mixture in a first physical state (typically either a gas phase or a liquid phase) is flowed across a column matrix in a second physical state (typically either a liquid phase or a solid phase) to separate a component of the mixture from other materials of the mixture. Accordingly, the desired component or components of the mixture must be retained preferentially by the matrix while the matrix remains physically separable from all or most of the undesired mixture components.

It can be desired to quantitate and/or otherwise analyze an amount of a component retained by a column matrix in a packed column. Accordingly, it can be desired to extract a retained component from a matrix material. A method of extracting a retained component is to subject the column matrix to conditions which disrupt interactions

between the matrix material and the component to thereby elute the component from the matrix material. In some applications, it is desirable to elute the retained material from the matrix material while the matrix material is still within a packed column, and in other applications it is desirable to remove the matrix material from a packed column before eluting the retained component. Additionally, there are some applications in which it is desirable to remove a matrix material from a packed column and thereafter analyze the matrix material directly to quantitate and/or otherwise analyze an amount of a component retained on the matrix material.

A difficulty in utilizing column-based separations is in removing matrix material from a column chamber and subsequently repacking additional matrix material in the chamber to re-form a packed column. There are numerous reasons for removing matrix material from a chamber. For instance, a matrix material of a packed column can be rendered unusable after an initial separation, or after an initial series of separations. A matrix material can be rendered unusable if it is degraded by fluids passed through the material during a separation. Also, the matrix material can be rendered unusable if it becomes contaminated by materials within a sample because such contamination can pose a risk of cross-contamination.

For one or more of the above-discussed reasons, it is frequently desirable to repeatedly pack and unpack a column chamber with matrix material. Because packing and unpacking of column chambers is a time-consuming and laborious process, disposable columns are generally used. However, disposable columns still require

manual or robotic labor for column changeout. Accordingly, it is desirable to develop new methods for packing and unpacking column chambers.

A recent improvement is described with reference to an apparatus **30** in **Figs. 2** and **3**. Referring to **Fig. 2**, apparatus **30** comprises a tubular column chamber **32** having an inlet end **34** and an outlet end **36**. Outlet end **36** terminates in close proximity to a plate **38**. Plate **38** can comprise a window configured to enable light to pass through for spectroscopic measurement of materials eluting from column chamber **30**. A matrix material **40** forms a packed column **42** within column chamber **32**. Packed column **42** has a lateral periphery defined by tubular chamber **32**. Packed column **42** can be formed by flowing a slurry of matrix material **40** and a carrier fluid into column chamber **32**. Outlet end **36** of column chamber **32** is displaced from plate **38** by a distance "D" sufficient to enable the carrier fluid to pass between column chamber **32** and plate **38**. However, the distance is less than an average width of matrix material **40**. Accordingly, matrix material **40** is retained in column chamber **32** and stacks against plate **38** to form packed column **42**.

Fig. 3 illustrates a system **30** for removal of matrix material **40** from packed column **42**. Specifically, column chamber **32** is raised to enable matrix material **40** to pass beneath column chamber **32** and over plate **38**. Subsequently, a fluid is flowed through chamber **32** to push matrix material **40** out of column chamber **32**.

System **30** is improved relative to other methods of packing and unpacking columns in that it can provide a quick method for releasing packed column material from

a column chamber, and can also provide a quick method for resetting the column chamber to be repacked with fresh matrix material. Most importantly, no permanently installed porous material is used to retain matrix material in a fluid stream. Porous material such as filter **22** in column **10** can clog by embedding relatively small particles from the matrix or mixture. A difficulty with column system **30** is that it can be problematic to move an entirety of column chamber **32** during transitions between packing and unpacking operations. Further, precise alignment is required to hold beads in the column. Discharged beads can undesirably pass through a detector. It can become increasingly difficult to move the entirety of column chamber **32** as a column-based separation is scaled up for larger operations. Accordingly, it is desirable to develop alternative methods for conveniently packing and unpacking column chambers, wherein a column chamber is not moved in transitioning between packing and unpacking operations.

An alternative embodiment is shown in **FIG.'s 4a, 4b** using an axially moveable solid rod **400** instead of the column chamber **32**. In this alternative embodiment, the tolerance between the outside dimension of the rod **400** and the inside dimension of the column chamber **32** is one half a bead diameter. A disadvantage of this embodiment is that the inserted portion or surface is alternately wetted and not wetted as it is inserted and withdrawn. A similar, alternately wetted and not wetted surface exists for column chamber **32 (FIG. 3)**. These inserted surfaces provide an opportunity for sample carryover into a subsequent sample. This is especially critical for DNA analysis wherein

a very small carryover from a previous sample can be detected in a subsequent sample. A related concern is that relatively small, abrasive particles may abrade or grind the alternately wetted and not wetted surface within the tightly pressed interface during actuation.

SUMMARY OF THE INVENTION

This invention is an apparatus and method of packing and unpacking a column chamber by alternately retaining and releasing a packed column in a fluid stream. A mixture of a fluid and a matrix material are introduced through a column chamber inlet so that the matrix material is packed within a column chamber. Once packed, the column may be used for purposes including but not limited to chemical extraction, titration, chemical sensing, filtration or combinations thereof. Uses generally involve the flow of a fluid mixture or carrier through the retained, packed column. After use, the matrix material is unpacked from the column chamber without removing the column chamber. More specifically, the column chamber having the column chamber inlet or first port for receiving the mixture further has an outlet port which is alternately restricted or open with respect to matrix material but always open with respect to fluid flow. When restricted, matrix material is retained; and when unrestricted or open, matrix material is released so that it can be ejected fluidically from the column chamber. The outlet port is alternately restricted or unrestricted with respect to the matrix by rotation relative one to the other of a rod with a binary end placed in the outlet port.

An advantage of the rod with the binary end is that the surface area of the rod in contact with fluid is always in contact with the fluid stream or the interior of the column chamber. In other words, there is no rod surface area that alternately contacts fluid then, say, an interior surface of the column chamber. This feature minimizes the potential of sample to sample contamination since a sample may be completely washed through and not captured on an alternately or intermittently exposed surface. This is especially valuable for nucleic acid or DNA samples wherein one molecule of a previous nucleic acid sample can be detected in a subsequent nucleic acid or DNA sample. A related aspect of this feature is the absence of any surface which is alternately a press-fitted interior surface and a fluid-contacting surface. This feature precludes the potential for abrasion by relatively small matrix or mixture particles carried by the alternating surface.

Isolation of nucleic acids from environmental samples such as soil is especially challenging because the co-extraction of metals, chelators, humic acids and other organic contaminants will interfere with downstream molecular biology procedures such as polymerase chain reaction (PCR). An advantage of the present invention is avoidance of co-extraction by using affinity matrix microparticles that selectively retain nucleic acids of interest but exclude all other soluble soil constituents.

The rod with a binary end also satisfies the need to alternately retain and release matrix material in a fluid stream without resorting to any permanently installed filter material which can clog by embedding particles.

The subject matter of the present invention is particularly pointed out and

distinctly claimed in the concluding portion of this specification. However, both the organization and method of operation, together with further advantages and objects thereof, may best be understood by reference to the following description taken in connection with accompanying drawings wherein like reference characters refer to like elements.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the invention are described below with reference to the following accompanying drawings.

FIG. 1 is a diagrammatic, cross-sectional view of a prior art column construction.

FIG. 2 is a diagrammatic, cross-sectional view of a prior art system for packing and unpacking a column chamber. The system of Fig. 2 is shown with the column chamber in a position for packing a matrix material within the column chamber.

FIG. 3 is a view of the Fig. 2 system, with the column chamber shown in a position for unpacking the column chamber.

FIG. 4a is a view of a prior art column chamber controlled with an inserted rod that is axially actuated, closed position.

FIG. 4b is a view of a prior art column chamber controlled with an inserted rod that is axially actuated, open position.

FIG. 5a is a view of an embodiment of the present invention having a rod for controlling matrix material flow, closed position.

FIG. 5b is a view of an embodiment of the present invention having a rod for

controlling matrix material flow, open position, chamfered rod rotated.

FIG. 5c is a view of an embodiment of the present invention having a rod for controlling matrix material flow, open position, column chamber rotated.

FIG. 5d is a view of a rod with a stepped binary end.

FIG. 5e is a top view of a straight stepped binary end.

FIG. 5f is a top view of a cup stepped binary end.

FIG. 5g is a top view of a yin-yang stepped binary end.

FIG. 5h is a cross section of a through wall hole chamfer.

FIG. 5i is a cross section of a wall to end hole chamfer.

FIG. 5j is a view of a concave stepped end.

FIG. 5k is a view of a convex stepped end.

FIG. 5l is a view of a multi-stepped end.

FIG. 6 is a cross section of a block supported system with temperature control.

FIG. 7a is a cross section of an alternative embodiment in the closed position.

FIG. 7b is a cross section of an alternative embodiment in the open position.

FIG. 8a is a cross section of a side penetrated embodiment in the closed position.

FIG. 8b is a cross section of a side penetrated embodiment in the open position.

FIG. 9 is a schematic view of a sample treatment apparatus of the present invention.

FIG. 10 is a graph of temperature versus time demonstrating precise temperature control for the present invention.

FIG. 11 is a PCR dilution series of the eluant obtained from the automated DNA purification from a spiked salt solution (top) and a spiked soil extract (bottom) using the temperature controlled renewable column system shown in FIG. 9, and the procedure described in Tables 1-4. Gel electrophoresis with ethidium bromide staining was used to detect the PCR product. Concentrate indicates a 2-fold concentration of the eluant, and dilutions of this concentrate are indicated in each lane.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention encompasses systems for column based separations configured to pack and unpack column chambers using a rotateable rod with an asymmetric end for trapping and releasing matrix material. Referring to Figs **5a, 5b, and 5c**, a system **50** for column-based separations comprises a column chamber **52** having an inlet end **54**, an outlet end **56**, and an actuator end **57**. Column chamber **52** comprises a longitudinal axis "X", and can be formed of, for example, glass, plastic or metal. In the shown cross-sectional sideview (**Fig. 5a**), column chamber **52** comprises a pair of opposing sidewalls **53** and **55**. Although sidewalls **53** and **55** are shown as physically separated in the shown cross-sectional view, it is to be understood that sidewalls **53** and **55** can be portions of a continuous periphery. For instance, column chamber **52** can have a cylindrical shape, with sidewalls **53** and **55** forming portions of a continuous circular periphery of the cylinder.

Outlet end **56** is alternately partially obstructed and opened with a rod **58** having a binary end **51** which is inserted into the actuator end **57**. The rod **58** has a surface that is non-absorbent of the sample. Possible rod materials include but are not limited to metal, plastic, plastic coated metal and combinations thereof. The rod **58** has a first end or binary end **51** and a second end **59.** By "binary" we mean that the rod end has an asymmetric feature with respect to a rotation about its longitudinal axis such that a

restriction gap is present in one angular rod orientation and an unrestricted gap in another orientation. The restriction gap is small enough so that matrix material cannot pass but large enough so that fluid can pass. Infinitely many shapes constitute such a binary end of which the preferred embodiment is the simplest geometry (shown in **Fig. 5a, 5b, and 5c**). Other example binary rod include but are not limited to the geometries are shown in **Figures 5d-5l** and combinations thereof. In all cases, the rod **58** is inserted into an actuator port **57** to a position wherein the binary end **51** of the rod **58** is situated at the intersection of the column chamber **55** and outlet **56;** and the second end **59** extends beyond the actuator port **57** where it may be connected to a mechanical actuator (preferred), or operated by hand.

The size of the annulus between the outside surface of the rod **58** and the inside surface of the outlet **56** that allows fluid flow but traps the matrix material **40** when the rod is in the closed position (**Fig. 5a**) is controlled by either adjusting the diameter of the rod, or by adjusting the column diameter. In the preferred embodiment shown in **Fig. 5a**, the diameter of the column inlet **54** is slightly larger than the diameter of the rod **58**, so that the annulus is about 20-30 micrometers. The matrix material **40** is preferably in the form of beads that are larger than the annulus. In preferred methods of the present invention, a slurry comprising a liquid carrier fluid and a matrix material is injected into column chamber **52**. The liquid carrier fluid then flows through outlet **56**, while the matrix material is retained by the chamfered end **51** of rod **58.**

The system **50** described with reference to Figs **5a, 5b, and 5c,** can be shifted from a packing mode to an unpacking mode by activating (rotating) either the rod **58** or the column chamber **52** with respect to the other. Specifically, when rod **58** is in a closed position, system **50** is in a column chamber packing mode (**Fig. 5a**), and when rod **58** is in an open position, system **50** is in a column chamber unpacking mode (**Fig. 5b**). During discharge of matrix material, a fluid, preferably a liquid, is flowed through the column chamber to flush matrix material from column chamber **52**. The fluid flowed during discharge of matrix material can be referred to as a dislodging fluid, and can be the same

as the carrier fluid.

In **Fig. 6**, a support structure **60** is provided to system **50**. Support structure **60** can comprise, for example, a plastic material molded or machined to fit system **50**. Alternatively, support structure **60** can include, for example, a clamp. The support structure **60** may further include a heater **62** for temperature control of the sample. The heater may be any heater including but not limited to fluid heat transfer (jacket), electric resistance heater, combustion heater and combinations thereof. A preferred heater is a resistance heater of stainless steel tube wrapped with Teflon (polytetrafluoroethylene) tape and wound with a nichrome wire encapsulated by a layer of thermally conducting, electrically insulating epoxy.

Temperature control may be a critical feature for sample handling of biomolecules including but not limited to DNA, protein and combinations thereof. Elevated temperature can help to purify a sample by excluding interferents either during analyte extraction from a sample or during a subsequent wash step. For example, the perfectly matched DNA sequence of a target DNA provides one of the strongest binding interactions between the matrix material and a component of the sample. Elevated temperature challenges binding of sample components so that only the strongest binding interactions, like that of the target DNA, succeed. Further, an increase in temperature above the capture temperature may be used to elute biological molecules from the matrix material. Heating to a temperature above the capture temperature changes the conformation (shape) of biomolecules and affects the binding equilibrium of ligands with the biomolecules. Hence, the increased temperature may be used, for example, to remove ligands from antibodies, remove ligands from receptors, and remove DNA from complementary DNA or DNA chimeras (e.g. PNA).

Because a metallic column chamber may introduce metal ions into the sample, it may be necessary to substitute a polymer, for example polyethyletherketone (PEEK) that is inert to the sample at the higher temperatures.

Once a nucleic acid sample is obtained by any of the above discussed procedures or by another procedure, it may be processed by polymerase chain reaction (PCR). The PCR may be carried out in a column chamber (with or without the rod) using the temperature control for thermal cycling for the PCR.

In addition to the preferred embodiments, the present invention encompasses further embodiments. For example in **Fig.'s 7a and 7b,** the outlet and inlet are reversed compared to **Fig.'s 5a, 5b and 5c.** In cases wherein the matrix material **40** is in the form of beads smaller than the annulus (for example PNA derivatized 20 micrometer Poros™), larger inert material (not shown), for example glass beads, may be inserted ahead of the matrix material **40** to capture the matrix material **40.**

The angle between inlet **54** and outlet **56** may be varied.

In **Fig. 8a** and **8b,** the rod **58** penetrates the side of the column chamber **50 rather** than the end of the column chamber **50.**

The column chamber is preferably packed and unpacked with matrix material while flowing fluids through the column chamber **50** in an identical flow direction during both the packing and unpacking operations. Specifically, all, or at least most, of the matrix material is preferably expelled from a column chamber along an identical flow direction as was utilized to pack the column chamber. However, it is to be understood that the invention also encompasses embodiments in which flow of fluid through a column chamber is reversed during a packing or unpacking operation. Preferably, the flow during a packing operation will not be reversed, but will instead be continuous in a flow direction from an inlet of the column chamber through an outlet. Also preferably, a flow of fluid through a column chamber will be predominately in a single direction from an inlet of the column through an outlet of the column during an unpacking operation. However, the unpacking operation can preferably also comprise some sporadic instances wherein flow is reversed (i.e., to go from an outlet to an inlet) to assist in dislodging matrix material from within column chamber.

Embodiments of the present invention can be operated with a vast number of

matrix materials, as will be appreciated by persons of ordinary skill in the art. Example classes of matrix materials include but are not limited to polymer, glass, metal ceramic and combinations thereof. Specific examples of matrix materials include but are not limited to sepharose, polystyrene, polyethylene glycol/polystryrene, zirconium, and hydroxyapatite as well as brand name resins such as POROS and Qiagen. Suppliers of materials for biological separations include but are not limited to Tepnel Life Sciences and PerSeptive Biosystems. Many of these resins can be derivatized to contain specific functional groups such as antibodies or oligonucleotides which can be used to bind specific biological materials_onto the resin material. Biological materials include but are not limited to viruses, both prokaryotic and eukaryotic cells, nucleic acids, proteins, other biomolecules and combinations thereof. Certain matrix materials are materials for specifically separating nucleotide fragments (e.g., nucleic acid, DNA, RNA or combinations thereof) based upon a sequence of the fragments, such as, for example, the Tepnel micro-beads discussed above in the Background section of this disclosure. The Sr resin, TRU-resin, and TEVA-resin described above in the Background section of this disclosure, can be utilized for separating radioactive molecules or atoms from other materials in the sample.

Nucleic Acid Separation and Purification: Apparatus and Methods

In an example method of the present invention, column **50 is** packed with a matrix material **40** that selectively binds nucleic acid fragments. Biological samples are prepared for separation on column **50** by lysing cells to form a lysis suspension. Insoluble debris is then removed from the lysis suspension by, for example, centrifugation and/or filtration to form a solution which is then passed through column **50** across the matrix material **40**. Nucleic acid fragments within the sample are then selectively bound to the matrix material **40**. Such nucleic acid fragments can be subsequently eluted from the matrix material **40** by methods known to persons of ordinary skill in the art, such as, for example, passing a solution comprising a low salt

concentration across the matrix material **40**.

An apparatus (**FIG. 8**) comprising a readily repackable column chamber **50** can have particular utility for purifying biological samples, as it is frequently difficult to completely regenerate a column **50** after such purification, and as the amount of material obtained is so small that even a minor amount of cross contamination can be problematic.

Example 1

PCR was used to evaluate the effectiveness of the separation and purification system for extracting a specific bacterial DNA (*Geobacter chapellii*) from a salt solution and also from a crude soil extract that contained a background of 10^8-10^9 genomes within 200µl, as well as humic acids and other organic material. When compared to manual benchtop extraction methods using the same reagents, the automated separation and purification system is faster (12 minutes versus several hours), and the extraction efficiency obtained using the automated extraction system is equivalent to benchtop methods using the same reagents.

Sequential Injection Analysis (SIA) is well known in the analytical chemistry literature as a pump and valve configuration and its associated fluid handling procedure for performing wet chemical analyses. **FIG. 9** diagrams the DNA extraction purification apparatus comprising a sequential injection system and the preferred embodiment temperature controlled column chamber **600**. The pumps and valves are preferably operated under computer control to automate the packing and disposal of the matrix material. Where only pumps and valves are used, the computer is capable of using a WINDOWS™ environment with preferred software FIALab™ for WINDOWS™ . However, addition of a stepper motor **900** for turning the rod, addition of heating elements for temperature control along with a thermocouple for measuring temperature are outside the scope of FIALab. Thus, the software needed to control an automated procedure may be written in any computer programming language, for example Visual Basic. FIALab commands are still incorporated into the preferred programming

environment by using an ActiveX™ version of FIALab. Procedural steps to implement the sequence and timing of operations are entered into the software.

A second stepper motor **902** may be used for controlling the syringe pump **904.** and selection valve were obtained as a unit from Alitea™ USA. The stepper motor controlled syringe pump **904** is connected to the common port of a selection valve **906** via a coiled length of tubing **908.** The coiled tubing (holding coil) **908** is used as a reservoir for holding a sample, extraction material or reagents. In each step of the sequential injection procedure, a liquid or slurry is aspirated into the holding coil **908** via a selected valve port, then the valve is switched to the column chamber port and the coil contents are injected into the column **600.** Air separators prevent bead slurry, sample and other solutions from mixing or dispersing.

As in prior art, a sequence of injections into the column chamber **600** packs the column chamber **600** with matrix material **40,** perfuses the matrix material **40** with sample and reagent, and then disposes of matrix material **40** to a detector or to waste **810.** For example, the first step in the preferred procedure is to aspirate matrix material slurry into the holding coil **908,** switch the valve **906** to the column chamber port and inject the slurry into the column chamber **600.** The binary end of the rod 58 in the column chamber **600** is in the closed position to retain the matrix material while allowing fluid to pass so that a packed column is formed. Similar back and forth motions of the syringe pump **904** coordinated with valve **906** selection allows for injection of sample, wash, eluent, and chemical cleaning agents as well as disposal of the used or spent matrix material. A recirculating pump **812** may be used for multiple passes of fluids through the column chamber **600.**

When used for DNA extraction, for example with DNA extraction solutions listed in Table 1, the automated DNA extraction procedure described above is used with the apparatus described above as outlined stepwise in Tables 2 through 4.

Table 1. Solutions used for DNA Extraction (FIG. 9 apparatus)

Matrix material slurry: 15mg /ml 1392r-Tepnel beads in 0.3 M NaCl
Hybridization solution: 0.2M NaP04, 0.1M EDTA, 0.25%SDS
Sample: a)200µl crude soil extract with 100ng *Geobacter metallireducens* DNA
or
b)200 µl hybridization solution with 100 ng *Geobacter metallireducens* DNA (sheared to 4-10 Kbp).
Wash solution: 0.5X SSC [0.15 M NaCl, 7.5 mM NaCitrate, pH 7.0]
Eluent: Water
Zap component 1**
Zap component 2 **

Tables 2-4 represent stages of one complete procedure which requires 12 minutes to complete. In order to make the procedure readable, standard implementation details were omitted, namely the use of 10 µL air separators between fluids, and loading of fluids into the holding coil prior to each injection. Table 2 lists the first sequence of injections into the flow cell ending with the elution of purified DNA. The flow cell is in the closed position throughout the Table 2 sequence; and the matrix material must be stirred into fluid suspension to create a "bead slurry" for the first injection. Tables 3 and 4 outline cleaning procedures after the extraction. All steps in Table 3 are performed at 50 µL/s. All steps involving DNA Zap reagents (Ambion, Inc. Austin, Texas) in Table 4 are performed at 10 µL/s except for water rinsing steps, which were 50 µL/s.

Table 4 refers to a set of stacked DNA Zap reagent zones. Throughout the preferred DNA extraction procedure, air separators keep fluids from mixing as they are aspirated into the holding coil and injected into the cell. However, in the Table 4 procedure air separators are omitted between the two DNA Zap reagents because these are deliberately mixed. When the two DNA Zap reagents are mixed, they form a short-lived intermediate chemical species that destroys DNA. Mixing is achieved in the sequential injection system by alternately aspirating short segments of each reagent into the holding coil. This is termed a "stacked zone" of reagents that are mixed by dispersion when flowed through tubing.

Table 2. Sequence of Injections into the Column Chamber for Matrix Packing and DNA Extraction (FIG. 9 apparatus)

solution	injection volume / µL	flow rate / (µL / s)	temperature / °C
slurry	113	10	(no heating)
hybridization	50	10	ramp up to 60

Nucleid acid sample solutions were heat denatured at 100 °C for 5 min. and quick-chilled on ice immediately before aspiration into the system.

sample	200	1	60
wash	60	3	45
	{stop flow. Ramp up to 85 °C}		
eluent	40	1	85

Table 3. Disposal of the Packed Matrix Material (FIG. 9 apparatus)

step 1: Aspirate 35 µL from the flow cell to pull matrix material particles away from the binary end of the rod.

step 2: Rotate the rod to the open position. Optional: aspirate another 20 µL to disperse the bead material into fluid suspension.

step 3: Eject the beads to waste by injecting 200 µL of water through the cell.

Table 4. Chemical Destruction of Residual DNA in the Flow System (FIG. 9 apparatus)

step 1: Aspirate stacked zones of DNA Zap reagent into the holding coil 10 µL further than the volume of sample used.

step 2: Push the DNA Zap reagent back out of the holding coil and into the (currently empty) sample inlet until it completely fills the sample inlet.

step 3: Switch the valve to the flow cell, and push the remaining DNA Zap reagent into the column chamber.

step 4: While the DNA Zap remains in the inlet and column chamber, aspirate another set of DNA Zap stacked zones into the holding coil.

step 5: Switch to the sample valve position and aspirate the previous DNA Zap mixture from the sample inlet into the holding coil behind the fresh mixture of DNA Zap. Again, aspirate to the point 10 μL further than the volume of sample used.

step 6: Inject all DNA Zap to waste through the column chamber.

step 7: Rinse the sample inlet and column chamber with water.

The matrix material used for the on-line affinity purification of DNA was obtained from Tepnel Life Sciences (Cheshire, England) and included universal 16S rRNA oligonucleotide 1392r with a dT8 linker (lower case) covalently attached to 60 μm polystyrene microbeads (bead- 5' ttttttttACGGGCGGTGTGTRC). The binding capacity was estimated to be 2 pmol mg^{-1} (or cm^2) beads (1.20×10^{12} probes mg^{-1}) based upon a competitive hot/cold assay using complementary oligonucleotides (Tepnel). This universal oligonucleotide sequence will capture all types of bacterial DNA on the column, and dilution to extinction PCR analysis may be used to specifically detect *Geobacter metallireducens* DNA that is eluted from the column.

The DNA extraction procedure outlined in Tables 2-4 requires temperature control of the column matrix **50**. **FIG. 10** shows 10 replicate thermocycles performed in the column chamber used for DNA extraction. This data was collected using a type-K thermocouple in the column chamber which was filled with aqueous solution. A feedback control algorithm achieved the sequence of targeted temperatures, 93, 65, and 72 °C. These temperatures and durations are suitable for PCR amplification of DNA.

Temperature control may be achieved during the DNA extraction without the intrusion of a thermocouple probe. This was done by first using intrusive measurement and feedback control to obtain a voltage vs time function that achieved the desired sequence of temperatures at the proper flow rates of the extraction procedure. The

resulting voltage vs time were recorded so that they were executed in future DNA extraction procedures as a substitute for feedback control. The temperature curves achieved in this way were found to be similar to those of feedback control.

A more robust method of temperature control is to use non-intrusive temperature measurement with feedback control. This can be achieved by using a resistance heater that also serves as a resistance temperature detector (RTD). For example, a resistance coil of nickel has a suitably high temperature coefficient of resistance. The temperature dependent coil resistance can be determined from the applied voltage and resulting current. The temperature can be determined in real-time once the calibration relationship is established between heater coil resistance and column temperature. The calibration points would be coil resistance and temperature data pairs over stable plateau regions such as those of **FIG. 10.**

Example 2

An experiment was conducted to demonstrate a DNA extraction according to the present invention. Genomic *Geobacter metallireducens* DNA was sheared to 4-10 Kbp in size by ballistic disintegration for 1 minute at 5000 oscillations s^{-1} in an 8-place bead beater (BioSpec Products, Inc., Bartlesville, OK) with 12 μg DNA, 0.75 g 0.1 mm glass beads and 500 μl water.

Nucleic acid extracts were prepared from a garden soil by aliquoting 12 x 0.5 g soil into 2.0 ml screw-cap microfuge tubes containing 1.5 g 0.1 mm glass beads and 1 ml extraction buffer [0.2M $NaPO_4$, 0.1M EDTA, 2% SDS, pH 8.0]. Slurries were frozen at -80°C for 1 hr, thawed at 65°C for 30 min. and cells lysed by ballistic disintegration at 5000 oscillations s^{-1} for 2 min. Glass beads, sediment and cell debris were removed by centrifugation at 14,600 x g for 10 min. at 18°C, and the supernatants pooled. Crude extracts were dialyzed against several changes of sterile water, passed through a 0.2 μm syringe-filter and the salt concentration adjusted to 0.3 M NaCl. The sodium ion concentration of the soil extract was adjusted to 0.3 M to provide solution conditions

comparable to those normally encountered in solution hybridization studies, standard membrane hybridizations and sequence-specific purification systems based on oligo-dT or biotinylated oligonucleotides and streptavidin-coated paramagnetic particles. Due to spectroscopic interference by humic acids and other soil constituents, total DNA in the crude soil extract was quantified by ethidium bromide staining after gel electrophoresis. Temperature control was achieved during the DNA extraction without the intrusion of a thermocouple probe.

The estimated nucleic acid concentration in 200 µl of crude soil extract was 3 µg, or 6 x 10^8 cell equivalents of genomic DNA.

FIG. 11 shows a PCR dilution series for the specific capture of *Geobacter metallireducens* DNA in salt solution (top) and a soil extract (bottom) using the automated system. The automated capture procedure is outlined in Tables 1-4. The eluant was lyophilized to dryness and resuspended in 20 µl of water prior to PCR. Since the salt solution used for the "Clean DNA" extraction does not contain PCR inhibitors, *Geobacter metallireducens* is detected by PCR at all dilutions shown in the top of **FIG. 11.** PCR inhibitors were present in the 2-fold concentrated eluant (FIG. 11), bottom, concentrate and concentrate + 200 fg spike), but not in the lanes with additional dilution **(FIG. 11,** bottom, 1:5, 1:25, 1:125). This is a positive result since it indicates that most of the PCR inhibitors are removed during the purification process, and therefore PCR can be used to detect DNA as long as the sample is not concentrated prior to PCR. Without purification, the *Geobacter metallireducens* spiked into the crude soil extract is not detectable by PCR.

The automated DNA extraction methods described in these reduction to practice experiments represent a significant improvement in processing time compared to manual DNA extraction procedures from soil. The *total* processing time for the automated extraction described above is 12 minutes, however, the manual extraction procedure typically requires 1 to 5 hours to complete (depending on the batch hybridization reaction time).

Radiological Separation and Purification: Apparatus and Methods

Although DNA extraction was used to demonstrate the utility of the apparatus for renewable column separation and purification described in this patent application, extraction procedures using packed column and renewable column prior art can generally be adapted using the present invention to provide additional renewable columns for chemical sample separation and purification according to the present invention. Chemical sample includes but is not limited to radioactive atom, chemical species, lipid and combinations thereof. The invention may specifically be used as a renewable column to separate radionuclides from nuclear waste samples. A proposed protocol is outlined in Tables 5 through 7, and a proposed system is as shown in **FIG. 8** absent the recirculating pump and absent temperature control. It is preferred that the column height of matrix material be increased for radiological separation. The present invention would be advantageous when using samples and/or affinity matrix materials that contain particulates that might clog a frit.

Table 5. Packing of Americium Extraction Column

Step#	Event (Flow Rate)
1.	Aspirate 100 µL of air into the holding coil (15 mL/min)
2.	Aspirate 635 µL of carrier into a syringe (35 mL/min)
3.	Aspirate 700 µL of sorbent slurry into the holding coil (3 mL/min)
4.	Dispense 700 µL of sorbent slurry to column chamber via the packing line (3mL/min)
5.	Repeat steps 4 and 5 three times
6.	Dispense 635 µL to the packing line (3 mL/min). Pause 12 seconds.

Table 6. Extraction Purification Sequence for Americium

Step#	Description: Reagent (Flow Rate)
2.	Condition column: 1.5 mL 2M HNO_3 (1.0 mL/min)
3.	Load sample: Inject 100 µL sample (1.0 mL/min)
4.	Wash column: Inject 6 mL 2M HNO_3 (1.0 mL/min)
5.	Switch the two-way valve to "detector"
6.	elute Am: 4 mL 3M HCl (1.0 mL/min)
7.	elute actinides: 4 mL 0.1M ammonium bioxalate (1 mL/min)

Table 7. Disposal of the Americium Extraction Column

Step#	Event (Flow Rate)
1.	Switch the two-way valve to the "save beads" position, and switch the selection valve to the "packing" position
2.	Aspirate 150 µL through the closed column chamber to move beads away from the binary end of the rod (12 mL/min)
3.	Open the column chamber and aspirate another 150 µL to disperse beads into fluid suspension (12 mL/min)
4.	Inject 1000 µL of water through the column chamber to eject beads to the "save beads" container

Closure

While a preferred embodiment of the present invention has been shown and described, it will be apparent to those skilled in the art that many changes and modifications may be made without departing from the invention in its broader aspects. For example, a second outlet (not shown) may be added below the binary end so that the second outlet is a fluid outlet only. The original outlet in cooperation with the binary end permits packing and unpacking of the matrix material. The appended claims are therefore intended to cover all such changes and modifications as fall within the true spirit and scope of the invention.

CLAIMS:

1. A method of packing and unpacking a column chamber, comprising the steps of :

flowing a mixture of matrix material and fluid into a column chamber and forming a packed column from the matrix material, said column chamber having a first port for receiving said mixture, an outlet port and an actuator port;

partially closing said outlet port for capturing said matrix material and permitting said fluid to flow therepast by rotating relative one to the other of a rod with a binary end placed in said actuator port and said column chamber; and

further rotating relative one to the other of said rod and said column chamber thereby opening said outlet and permitting said matrix material and said fluid to flow therethrough thereby unpacking the matrix material from the column chamber.

2. The method of claim 1 wherein the unpacking removes substantially all of the matrix material from the column chamber.

3. The method of claim 2 wherein all of the matrix material is removed from the column chamber.

4. The method of claim 1 wherein a first fluid is flowed into the column chamber during packing and a second fluid is flowed into the column chamber during unpacking, the second fluid being different from the first fluid.

5. A method of forming a packed column, comprising:

providing a column chamber, the column chamber having an inlet end and an outlet end, the outlet end having an actuator port and a flow port, the flow port alternately open or obstructed by a binary end of a rod placed in the actuator port, the flow port first obstructed with the binary end; and

flowing a mixture of the first fluid and the matrix material into the column chamber through the inlet end for packing the matrix material within the column chamber.

6. The method of claim 5 further comprising, after packing the matrix material in the column chamber, opening the flow port by a rotation of either of the rod or the column chamber with respect to the other and flowing a second fluid through the column chamber thereby unpacking the matrix material from the column chamber.

7. The method of claim 6 wherein the first fluid and the second fluid are the same.

8. A method of purifying a component of a sample, comprising:

providing a column chamber, the column chamber having an inlet end and an outlet end, the outlet end having an actuator port and a flow port, the flow port being partially obstructed with a rod with a binary end;

flowing the first fluid and the matrix material into the column chamber through the inlet end and along the first flow path to form a packed column of the matrix material within the column chamber, the rod holding the matrix material and permitting flow of the first fluid therethrough, the matrix material being configured to selectively retain a component of the sample;

flowing the sample through the packed column for separating the component from the rest of the sample;

unobstructing the flow port; and

flowing a second fluid through the column chamber to remove the matrix material from the column chamber.

9. The method of claim 8 wherein said sample is a chemical sample.

10. The method of claim 8 wherein the sample is a biological sample.

11. The method as recited in claim 10, wherein said biological sample has the component of a nucleic acid.

12. The method as recited in claim 10, wherein said biological sample has the component of a protein.

13. The method of claim 11 wherein the nucleic acid comprises at least one of DNA or RNA.

14. The method of claim 8 further comprising eluting the component from the packed column before removing the matrix material from the column chamber.

15. The method of claim 8 further comprising eluting the component from the matrix material after removing the matrix material from the column chamber.

16. The method of claim 8 further comprising recirculating at least some portions of the sample through the packed column prior to removing the matrix material from the column chamber.

17. A method of purifying a biological sample, comprising:

providing a column chamber, the column chamber having an inlet end and an outlet end, the outlet end having a flow port and an actuator port partially obstructed with a rod with a binary end;

flowing a mixture of the first fluid and the matrix material into the column chamber to form a packed column of the matrix material within the column chamber, the matrix material being configured to selectively retain a biological sample;

flowing a sample containing the biological sample through the packed column to separate the biological sample from other components of the sample;

flowing a second fluid through the column chamber to remove the matrix material from the column chamber.

18. A column-based separations system, comprising:

a column chamber having an inlet and an outlet, said outlet in fluid communication with a first flow path alternately obstructed and opened by

a rod with a binary end.

19. The system of claim 18, further comprising a column matrix material retained in said column chamber by said rod.

20. The system of claim 18, further comprising a heater in thermal contact with said column chamber.

ABSTRACT OF THE DISCLOSURE

In one aspect, the invention encompasses a method of packing and unpacking a column chamber. A mixture of a fluid and a matrix material are introduced through a column chamber inlet so that the matrix material is packed within a column chamber to form a packed column. After the packing, the matrix material is unpacked from the column chamber without moving the column chamber. More specifically, the column chamber having the column chamber inlet or first port for receiving the mixture further has an outlet port and an actuator port. The outlet port is partially closed for capturing the matrix material and permitting the fluid to flow therepast by rotating relative one to the other of a rod placed in the actuator port. Further rotation relative one to the other of the rod and the column chamber opens the outlet and permits the matrix material and the fluid to flow therethrough thereby unpacking the matrix material from the column chamber. In another aspect, the invention encompasses a method of purifying a component of a sample. A column chamber having an inlet end, an outlet end and an actuator end is provided. Flow of matrix material is obstructed by a rod with a binary end inserted in the actuator end. A suspension of the first fluid and the matrix material is flowed into the column chamber to form a packed column of the matrix material within the column chamber. The matrix material is configured to selectively retain a component of the sample. The sample is flowed through the packed column and past the rod to separate the component from the rest of the sample. The rod or the column chamber is rotated with respect to the other to open the outlet end and to remove the matrix material

from the column chamber. In yet another aspect, the invention encompasses a system for column-based separations. The system comprises a fluid passageway containing a column chamber and a flow path in fluid communication with the column chamber. The flow path is partially obstructed by a rod. The flow path extends through the column chamber and through the outlet end. The flow path is configured to form a packed column within the column chamber when a suspension of the fluid and the column matrix material is flowed along the flow path.

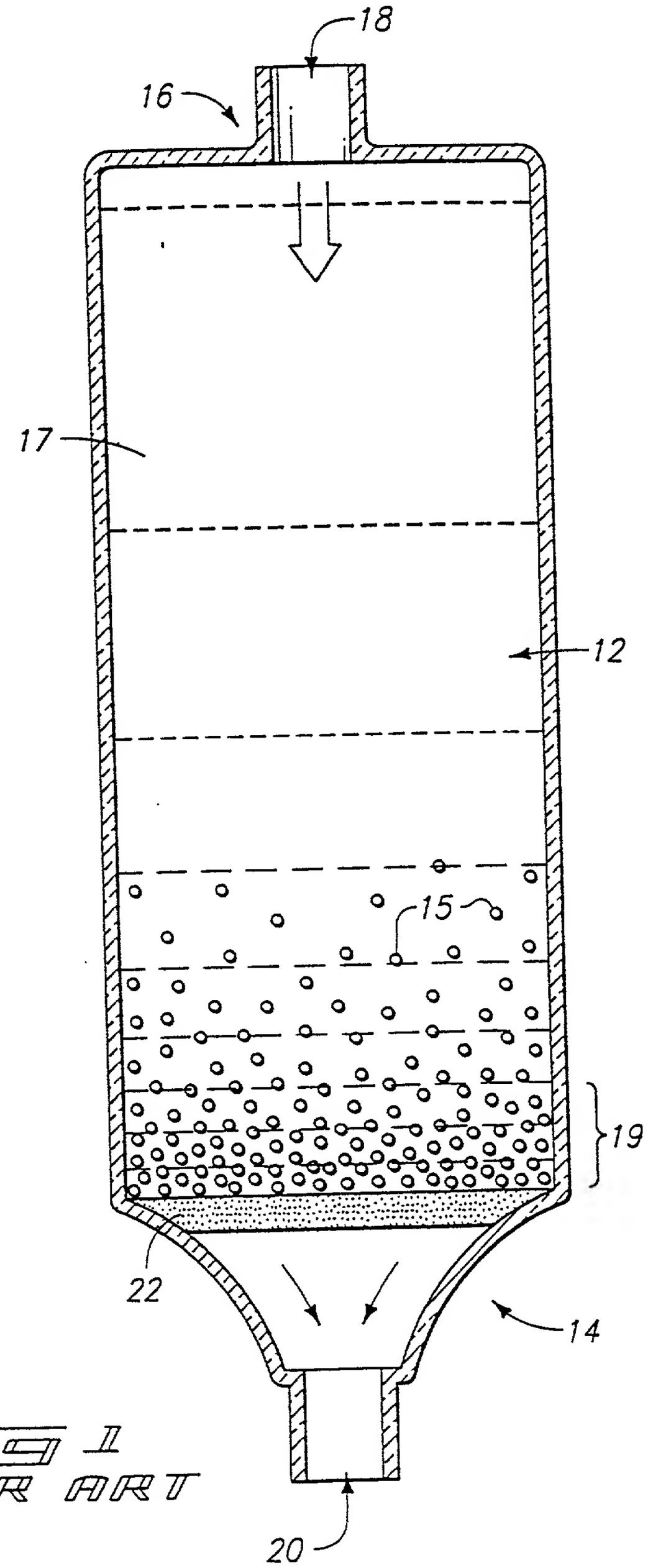

FIG 1
PRIOR ART

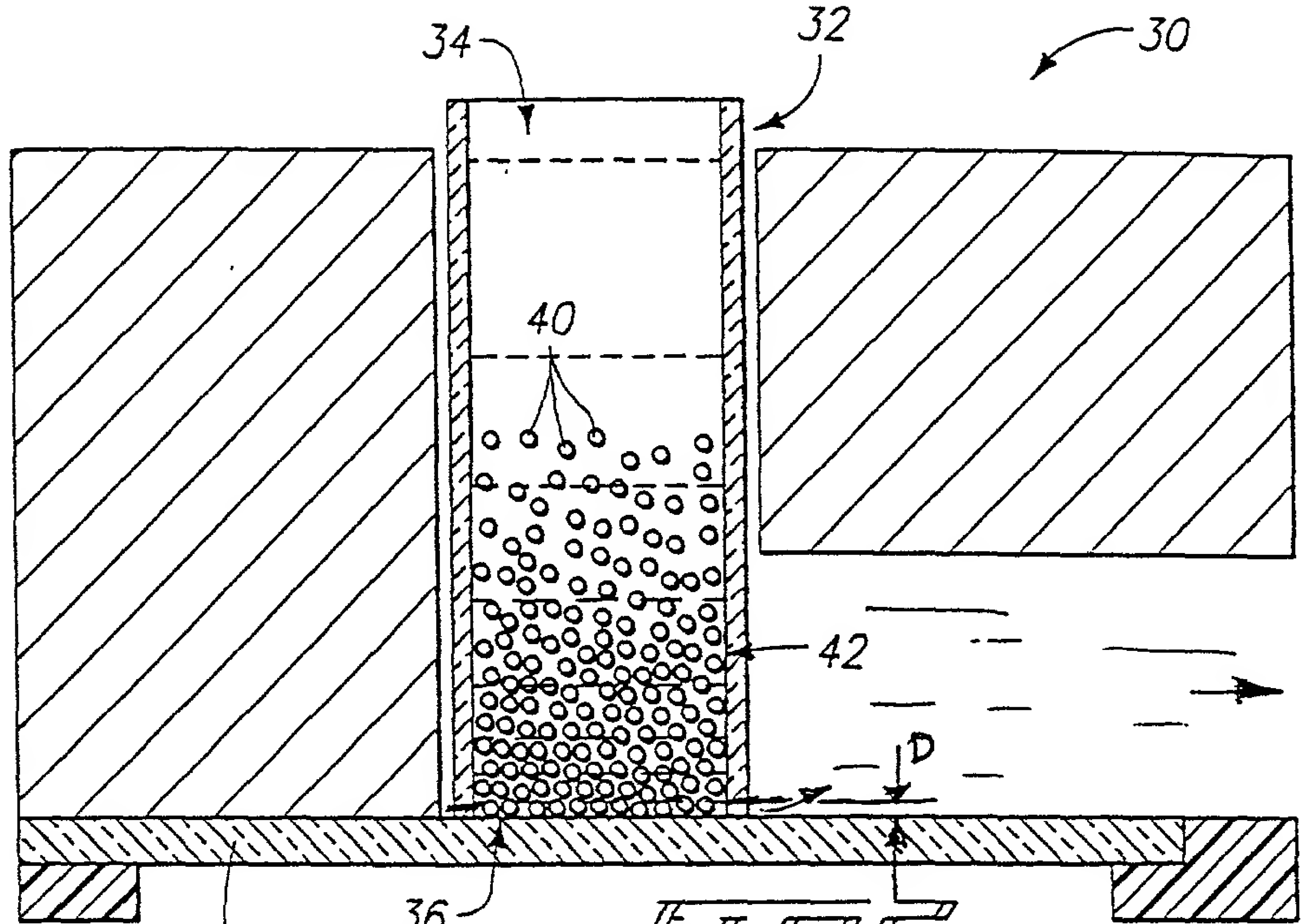

Fig 2
PRIOR ART

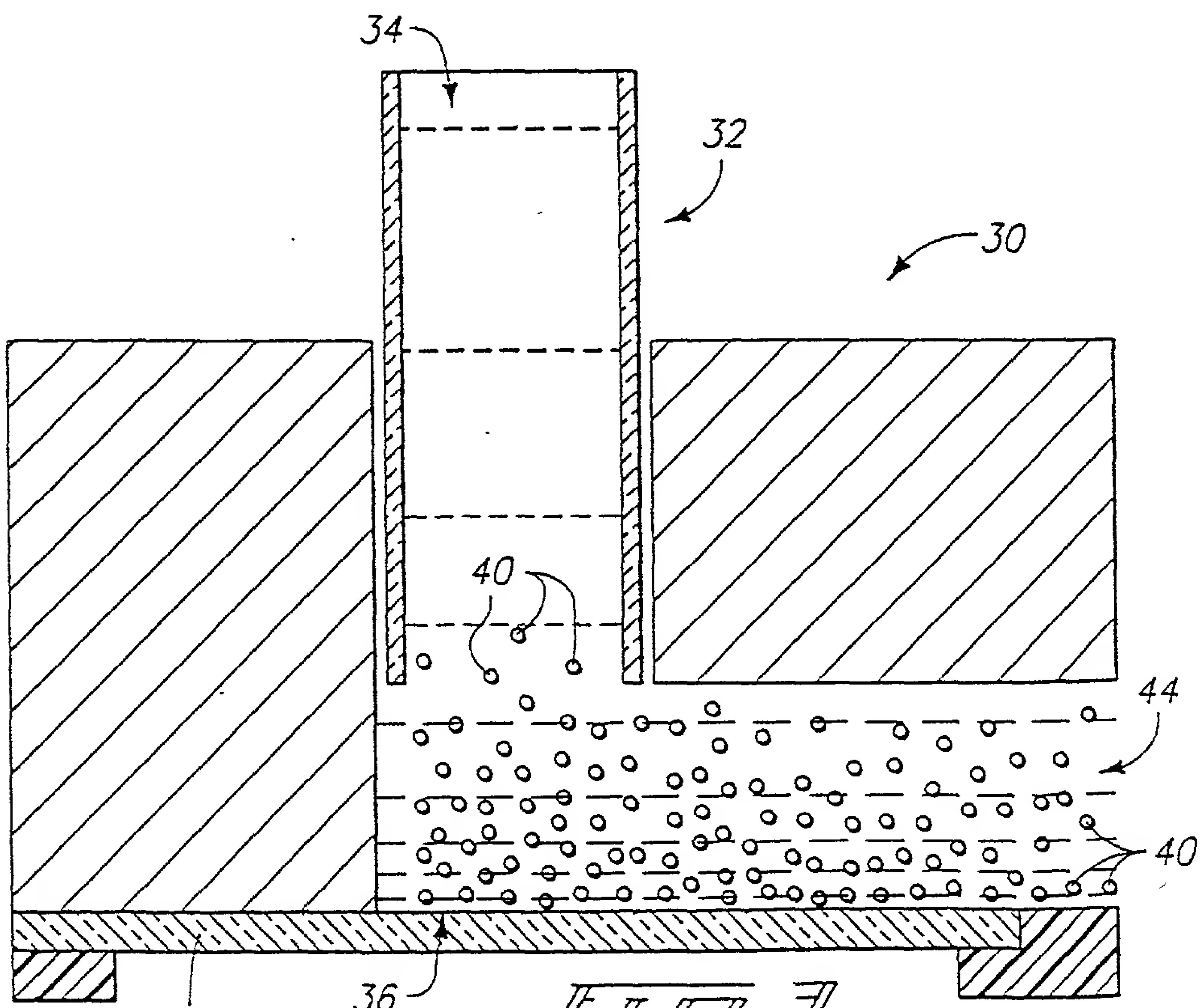

Fig 3
PRIOR ART

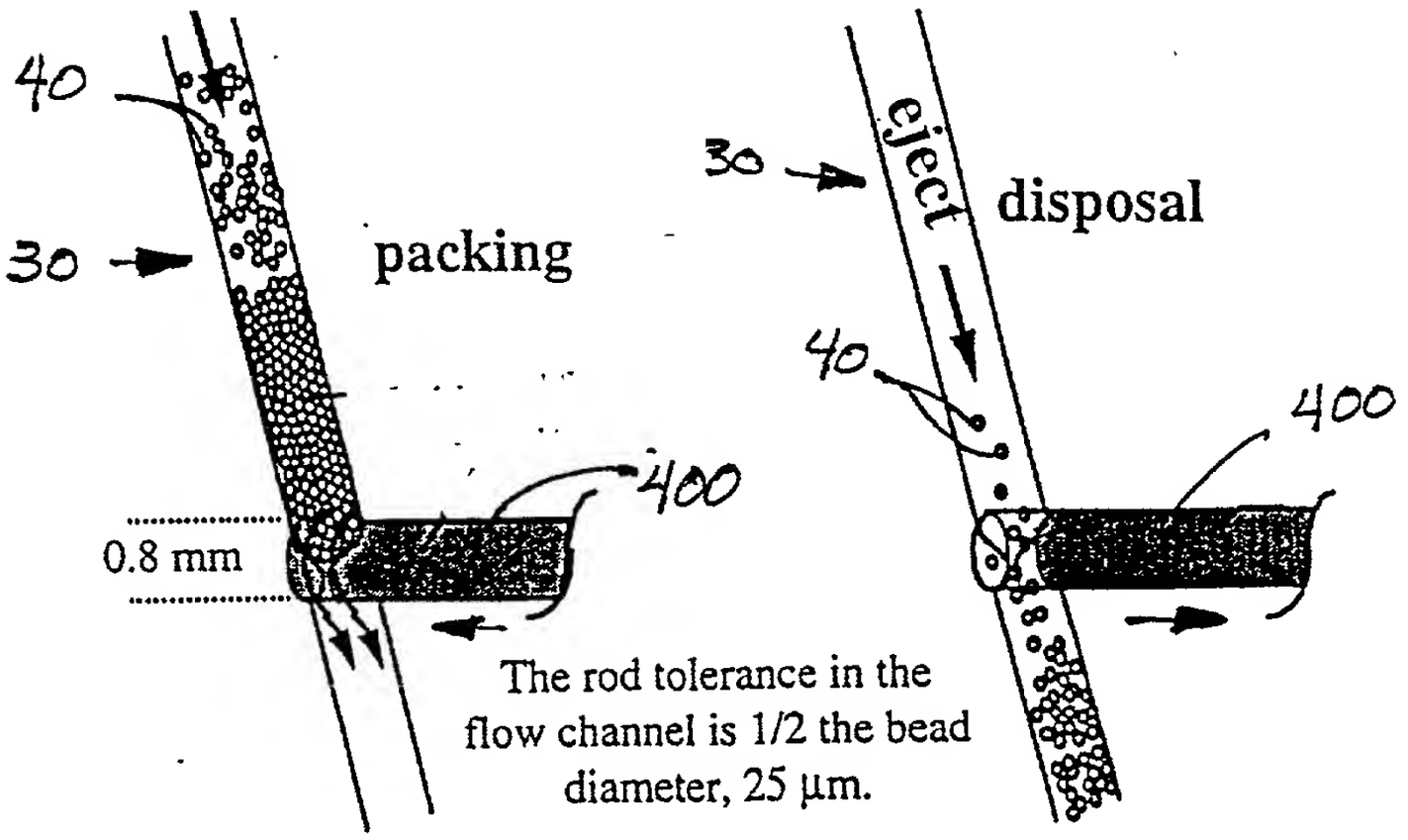

FIG. 4a (PRIOR ART)

FIG. 4b (PRIOR ART)

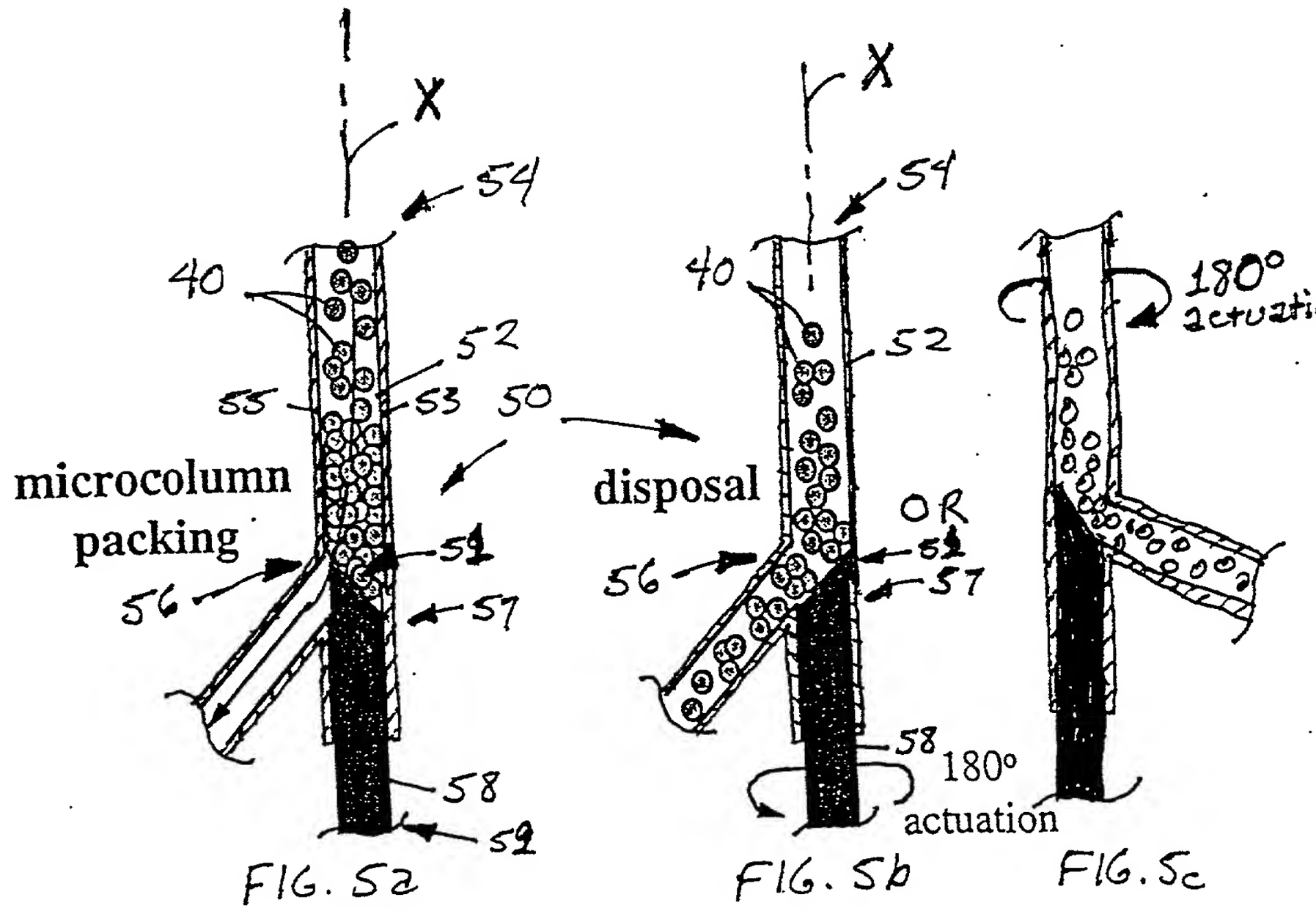

FIG. 5a

FIG. 5b

FIG. 5c

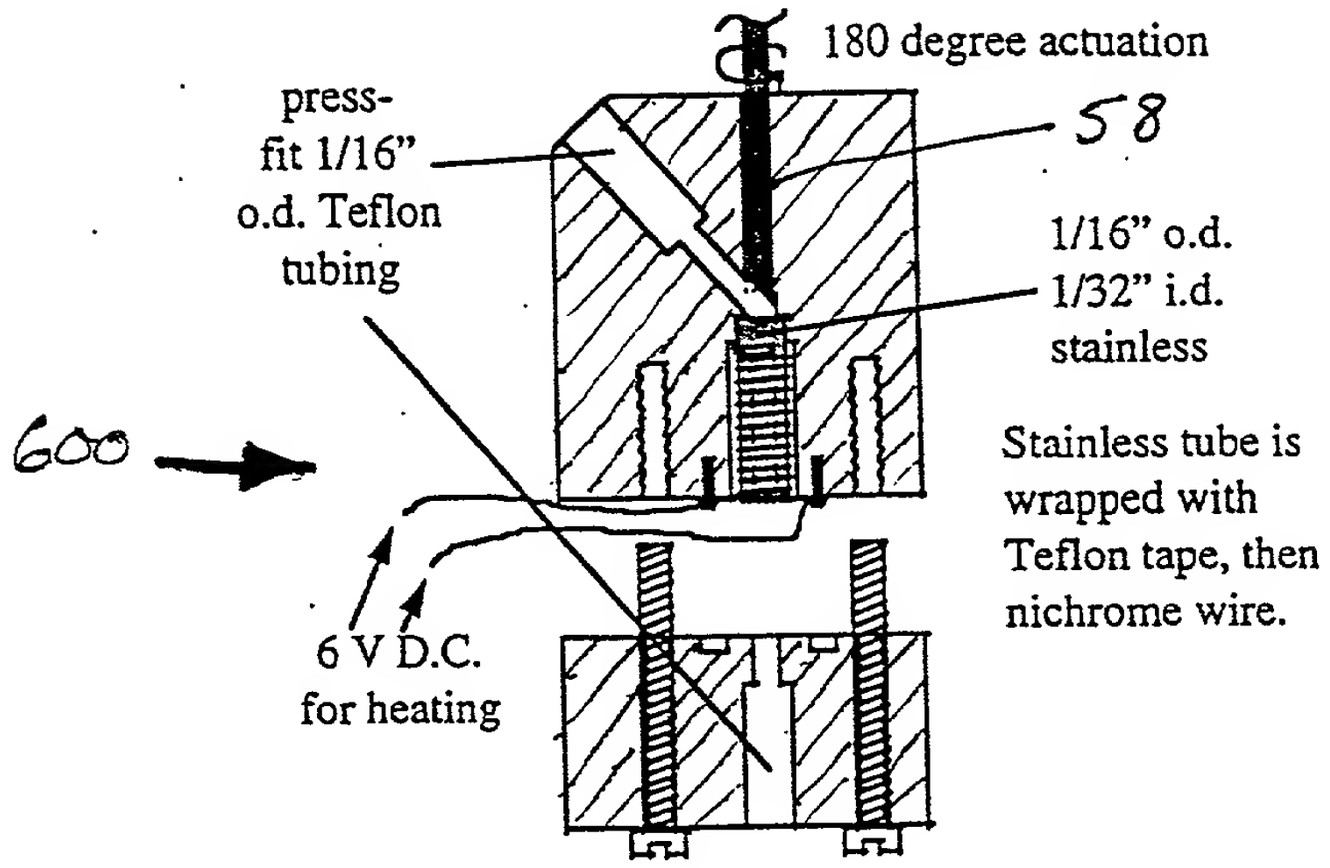
180 degree actuation
58
press-
fit 1/16"
o.d. Teflon
tubing
1/16" o.d.
1/32" i.d.
stainless
600
Stainless tube is
wrapped with
Teflon tape, then
nichrome wire.
6 V D.C.
for heating

FIG. 6

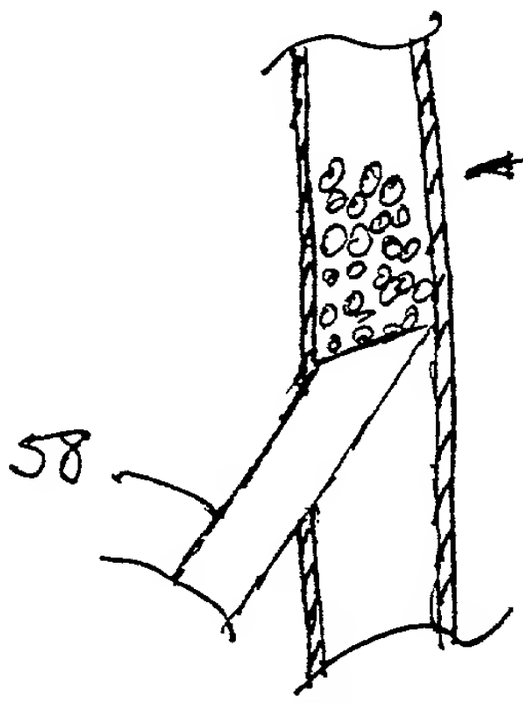

FIG. 8a

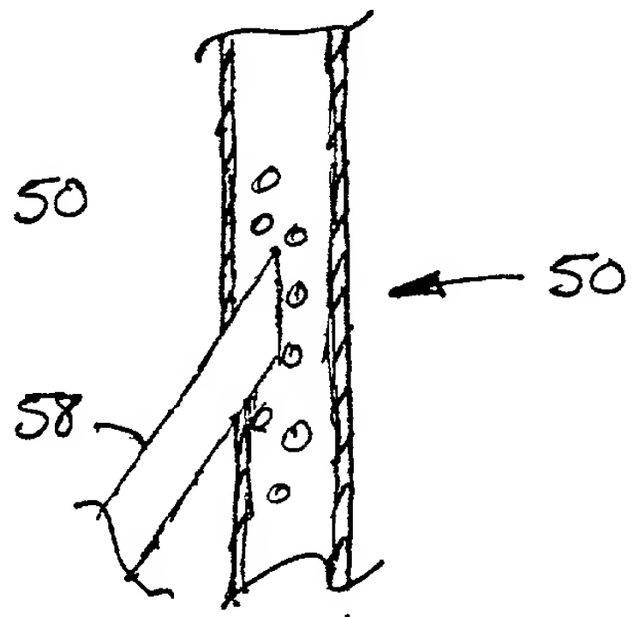

FIG. 8b

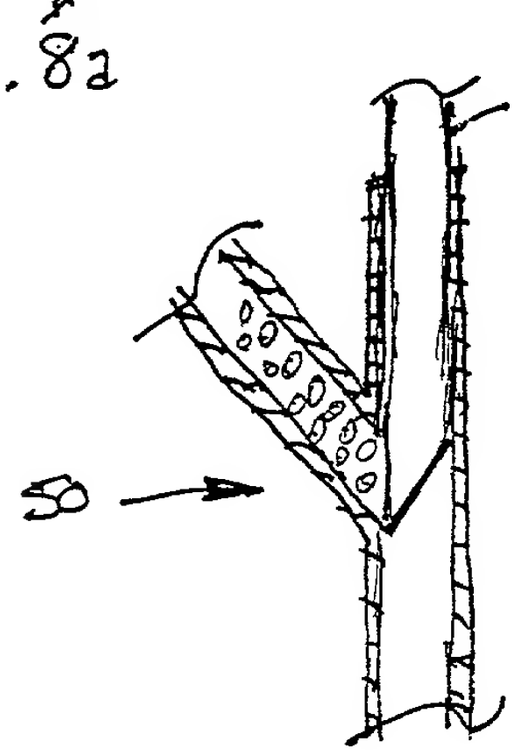

FIG. 7a

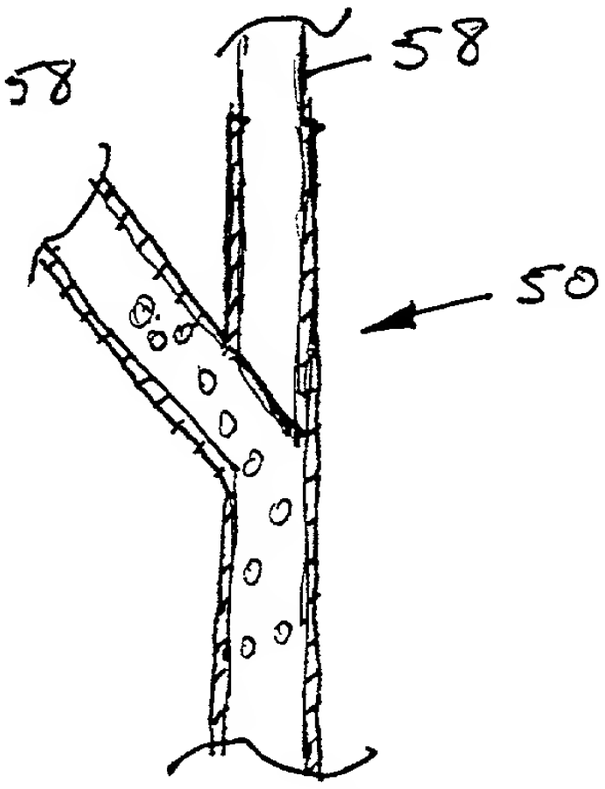

FIG. 7b

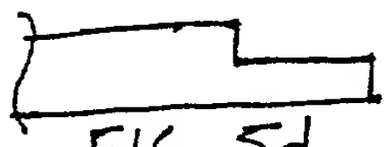

FIG. 5d

FIG. 5e

FIG. 5f

FIG. 5g

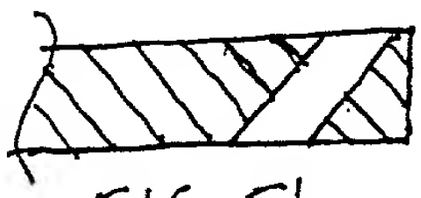

FIG. 5h

FIG. 5j

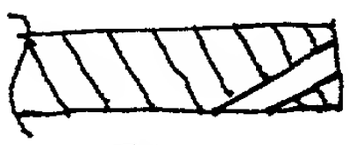

FIG. 5i

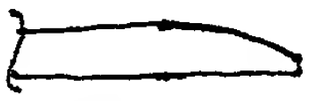

FIG. 5k

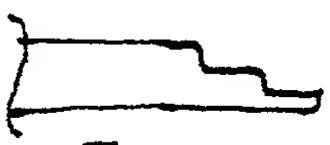

FIG. 5l

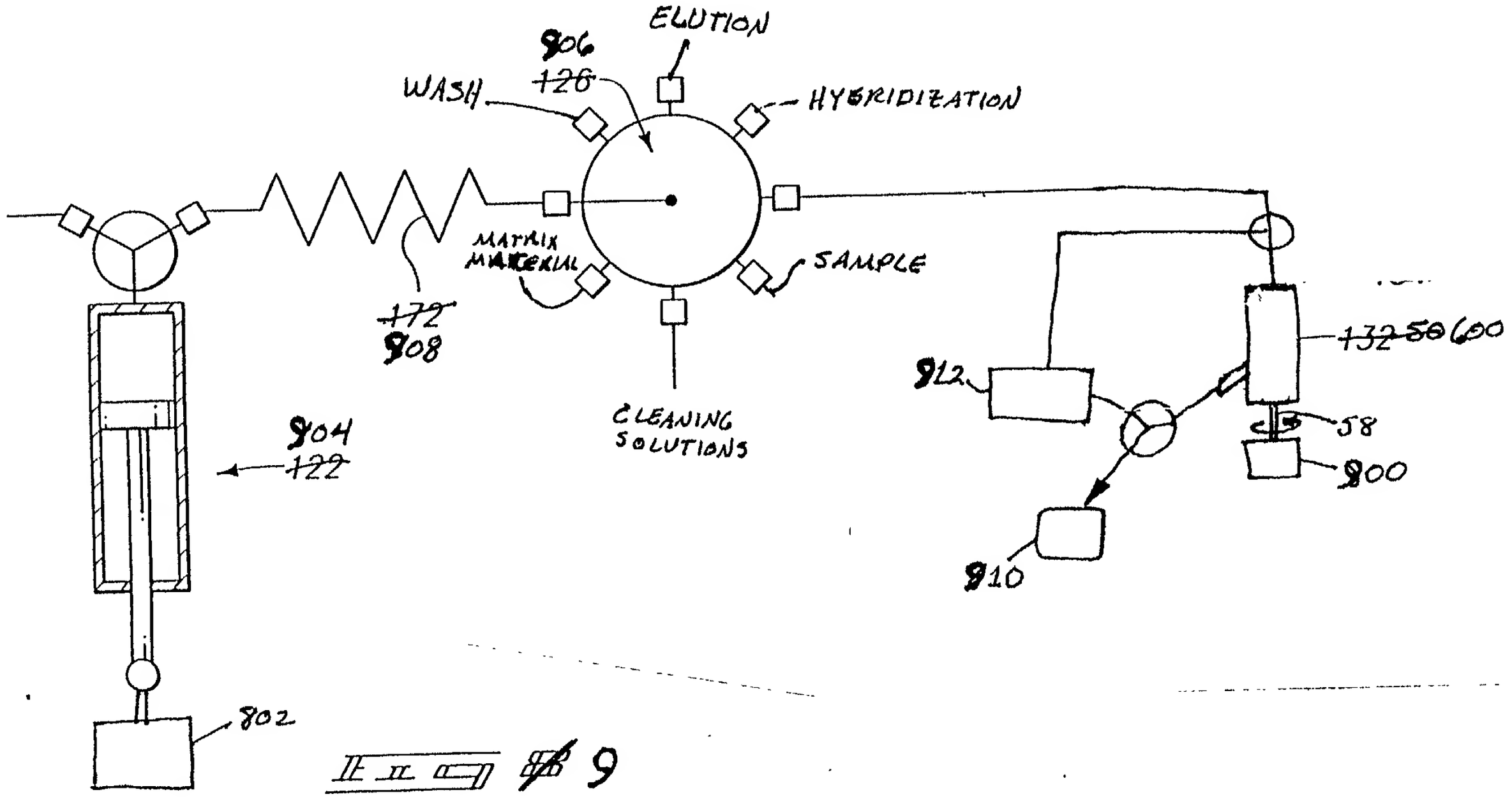

Fig 9

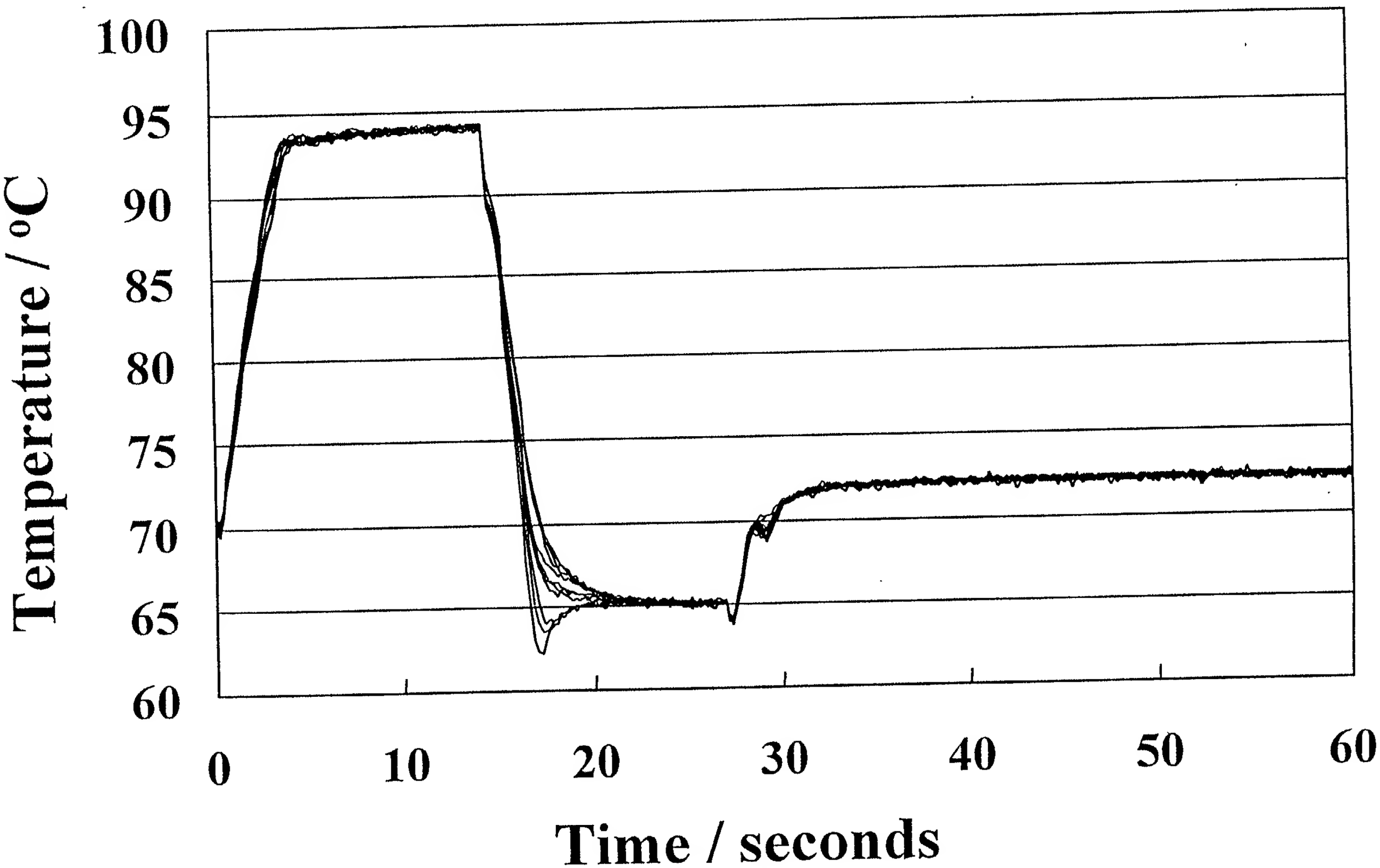

FIG. 10

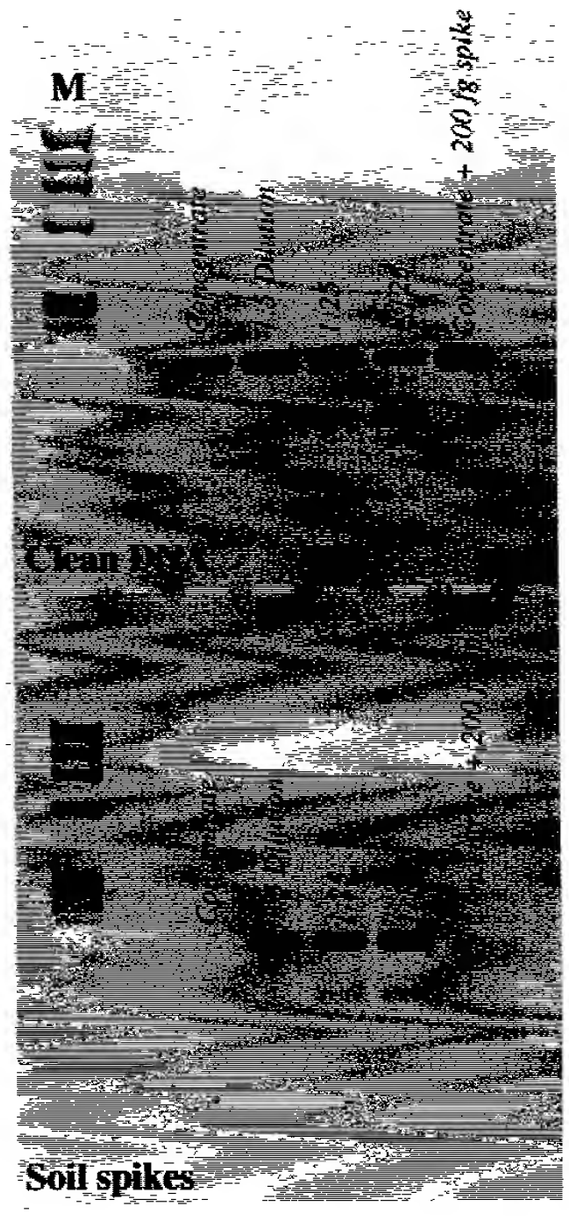

FIG. 11

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PATENT
File No. E-1658

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name,

I believe I am an original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD AND APPARATUS FOR PACKED COLUMN SEPARATIONS AND PURIFICATIONS, the specification of which

[x] is attached hereto.

[ ] was filed on _____ as
Application Serial No. _____

[ ] and was amended on _____
(if applicable)

[ ] with amendments through _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Sec. 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign
application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

[X] no such applications have been filed

[ ] such applications have been filed as follows

Prior Foreign Application(s)			Priority Claimed	
NONE				
(Number)	(Country)	(Day/Month/Year Filed)	[ ] Yes	[ ] No
NONE				
(Number)	(Country)	(Day/Month/Year Filed)	[ ] Yes	[ ] No

I hereby claim the benefit under Title 35, United States Code, Sec. 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Sec. 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Sec. 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

NONE		
(Application Serial No.)	(Filing Date)	(Status - patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

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Richland, WA 99352

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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